Symposium: Evolution & Computation to Design New Protein Functions

948-Symp
Designer Enzymes
Donald Hilvert.
Swiss Federal Institute of Technology, Zurich, Switzerland.
Protein design is a challenging problem. We do not fully understand the rules of protein folding, and our knowledge of structure-function relationships in these macromolecules is at best incomplete. Nature has solved the problem of protein design through the mechanism of Darwinian evolution. From primitive precursors, recursive cycles of mutation, selection and amplification of molecules with favorable traits have given rise to all of the many thousands of gene products in every one of our cells. An analogous process of natural selection can be profitably exploited in silico and in the laboratory on a human time scale to create, characterize and optimize artificial catalysts for tasks unimagined by Nature. Recent progress in combining computational and evolutionary approaches for enzyme design will be discussed, together with insights into enzyme function gained from studies of the engineered catalysts.

949-Symp
Computation-Guided Vaccine Design
William Schief[1,2].
[1]The Scripps Research Institute, La Jolla, CA, USA, [2]International AIDS Vaccine Initiative, New York, NY, USA.
Starting from crystal structures of virus-neutralizing monoclonal antibodies in complex with their epitopes, we employ computational design and in vitro screening of computation-guided mutagenesis libraries to engineer novel, minimal protein antigens that stabilize epitopes in their antibody-bound conformations and bind with high affinity and specificity to the target antibodies. We have also embarked on interface engineering to improve affinity for germline precursors of specific antibodies, and on developing multimeric platforms to heighten immune responses through avidity and other effects. The methods usually involve protein backbone manipulation and can be employed to control protein structure and interaction more generally.

Symposium: Imaging from Molecules to Organisms

950-Symp
Multiscale Imaging of Tissue Mechanics
Jan Liphardt.
Dept Physics, Univ California, Berkeley, Berkeley, CA, USA.
Many biological processes can only be fully understood by explicitly considering the mechanics of cells and tissues. For example, embryonic development requires millions of cells to change their size and shape, tensile properties, and contractility in an intricately choreographed manner. I'll describe our recent efforts to measure and manipulate the mechanics of developing and disorganizing multicellular structures. We have had some success with quantitative polarization microscopy as a tool for studying gradual changes in ECM organization. We have also synthesized photoactivatable collagen binding proteins, which allow fiducial grids to be optically written into developing structures and collagen fibers to be readily visualized.

951-Symp
Single Molecule Fluorescence and Optical Traps Applied to Molecular Motors: Two can do it better than One
Paul R. Selvin[1], Ben H. Blehm[1], Mindy Tonks-Hoffman[1], Kathleen M. Trybus[2], Trina A. Schroer[3], Christopher L. Berger[2], Ahmet Yildiz[4], Yann R. Chemla[1].
[1]University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]University of Vermont, Burlington, VT, USA, [3]Johns Hopkins University, Baltimore, MD, USA, [4]University of California, Berkeley, Berkeley, CA, USA.
Kinesin and dynein are molecular motors that move in opposite directions on a microtubule. They often act on the same cargo, causing the cargo to frequently switch direction. Whether this back-and-forth motion results from a coordinating complex or from a tug-of-war between the two motors is currently unknown. We have applied single molecule fluorescence to determine that they are undergoing a synergistic tug-of-war. By synergistic, we mean that the combination of the two motors is able to bypass roadblocks along the microtubule. When a motor is driven by kinesin, it approaches a roadblock (either other microtubules or microtubule-associated-proteins), is forced to turn around, relying on dynein. After a few tries, the dynein appears to step sideways onto another protofilament, at which point, when the kinesin takes over, it is able to bypass the roadblock. We also tested the tug-of-war model inside of a cell by using an in vivo optical trap. The in vivo optical trap is able to measure the stall forces in a viscoelastic media, which is present inside of a cell. By comparing directional stall forces in vivo and in vitro, we found that when cargo is going in the positive microtubule direction, kinesin and dynein are pulling, with the dynein walking backwards. The net stall force equals the stall force of kinesin ($\approx$ 7 pN) minus the stall forces of the number of dyneins (1.1 pN x ND, where ND, = 0 to 6). When moving in the negative microtubule direction, the stall force is just equal to a multiple of dynein's stall force (1.1 pN x ND), implying that kinesin has fallen off the microtubule.

952-Symp
Photoacoustic Molecular Imaging and its Biophysical Applications
Adam de la Zerda.
Stanford University, Berkeley, CA, USA.
Many cancer-specific biomolecules are dynamic in space, time and local environments. Hence, in order to truly understand their role in cancer progression, it is important to visualize them in living subjects - their most natural environment. In this invited talk, I will show how we utilize the 'photoacoustic effect' - the conversion of short light pulses into ultrasound waves, for performing highly sensitive disease detection in a living body. By measuring the ultrasound waves emanating from the body, one can create a detailed 3D image of the blood vessels structure, oxygen saturation levels and track external contrast agent molecules as they target diseased tissues such as cancer. I will present our experimental photoacoustic imaging system, the customized nanoparticle imaging agents we synthesized, and review a number of in vivo molecular assays we performed using this technology in cancer and eye diseases. Finally, I will present our work on a multi-modality nanoparticle technology that can visualize and guide the resection of a brain tumor along its true margins, including the tumor's finger-like protrusions that extend into the healthy brain.

953-Symp
Super-Resolution Fluorescence Imaging by Storm
Xiaowei Zhuang[1,2].
[1]Chemistry and Chemical Biology, Harvard Univ, Cambridge, MA, USA, [2]Howard Hughes Medical Institute, Cambridge, MA, USA.
Light microscopy is an essential tool in biological research. However, the spatial resolution of light microscopy, classically limited by diffraction to a few hundred nanometers, is substantially larger than typical molecular length scales in cells. Hence many subcellular structures cannot be resolved by conventional light microscopy. We recently developed a new form of super-resolution fluorescence microscopy, stochastic optical reconstruction microscopy (STORM), that breaks the diffraction limit. STORM uses single-molecule imaging and photo-switchable fluorescent probes to temporally separate the spatially overlapping images of individual molecules. This approach allows the localization of fluorescent probes with nanometer precision and the construction of sub-diffraction-limit images. Using this method, we have achieved multicolor and three dimensional (3D) imaging of live cells and tissues with nanometer-scale resolution. In this talk, I will discuss the recent technological advances and biological applications of STORM.

Symposium: Mechanics in the Nucleus

954-Symp
The RNA Molecule and its Processing by Molecular Motors
Nynke Dekker.
Delft University of Technology, Delft, Netherlands.
Single-molecule force and torque spectroscopy have shed much light on genomic processes such as transcription and replication. Here, we first use these techniques to shed light on the mechanical properties of RNA molecules. Then, by analogy with DNA where an understanding of its mechanical properties has formed the foundation for studying DNA-protein interactions, we study RNA-protein interactions, focusing on the activity of RNA-dependent RNA polymerases. We show how a very general approach that consists of parallel tracking to acquire hundreds of traces of individual RNA-dependent RNA polymerases transcribing RNA in real time combined with an analysis method that can simultaneously probe on-pathway and off-pathway events can readily elucidate the mechanochemistry of these enzymes. This approach is general and applicable to all kinds of DNA/RNA-protein interactions.